

Aberystwyth University

Effect of extruded linseeds alone or in combination with fish oil on intake, milk production, plasma metabolite concentrations and milk fatty acid composition in lactating goats

Bernard, L.; Leroux, C.; Rouel, Jacques; Delavaud, C.; Shingfield, Kevin; Chilliard, Y.

Published in:

Animal

DOI:

[10.1017/S1751731114003048](https://doi.org/10.1017/S1751731114003048)

Publication date:

2015

Citation for published version (APA):

Bernard, L., Leroux, C., Rouel, J., Delavaud, C., Shingfield, K., & Chilliard, Y. (2015). Effect of extruded linseeds alone or in combination with fish oil on intake, milk production, plasma metabolite concentrations and milk fatty acid composition in lactating goats. *Animal*, 9(5), 810-821. <https://doi.org/10.1017/S1751731114003048>

General rights

Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400

email: is@aber.ac.uk

RUNNING HEAD: Linseeds and fish oil on milk fat in goats

Effect of extruded linseeds alone or in combination with fish oil on intake, milk production, plasma metabolite concentrations and milk fatty acid composition in lactating goats

Laurence Bernard^{1*}, Christine Leroux¹, Jacques Rouel¹, Carole Delavaud¹, Kevin J. Shingfield^{2,3} and Yves Chilliard¹

¹*INRA, UMR 1213 Herbivores, Equipe Alimentation Génomique Lactation, Site de Theix, F-63122 Saint-Genès-Champanelle, France*

²*MTT Agrifood Research Finland, Animal Production Research, FI-31600, Jokioinen, Finland*

³*Institute of Biological, Rural and Environmental Sciences, Aberystwyth University, Gogerddan, SY23 3EE, United Kingdom*

*Corresponding author:

Laurence Bernard

Unité de Recherches sur les Herbivores

INRA-Theix

63122 St. Genès-Champanelle

France

Tel (+33) (0)473624051

Fax (+33) (0)473624519

Email: Laurence.Bernard@clermont.inra.fr

ABSTRACT

Based on the potential benefits for long-term human health there is interest in developing sustainable nutritional strategies for lowering medium-chain saturated fatty acids (FA) and increasing specific unsaturated FA in ruminant milk. Dietary supplements of extruded linseeds (EL), fish oil (FO) or a mixture of EL and FO increase *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and long-chain n-3 polyunsaturated FA in bovine milk. Supplements of FO cause milk fat depression (MFD) in lactating cows, but information for dairy goats is limited. Fourteen Alpine goats were used in a replicated 3 x 3 Latin square with 28 d periods to examine the effects of EL alone or in combination with FO on animal performance, milk fat synthesis and milk FA composition. Treatments comprised diets based on natural grassland hay supplemented with no additional oil (control), 530 g/d of EL or 340 g/d of EL and 39 g/d of FO (ELFO). Compared with the control, ELFO tended ($P = 0.08$) to lower milk fat yield, whereas EL increased ($P < 0.01$) milk fat content and secretion (15 and 10%, respectively). Relative to EL, ELFO decreased ($P < 0.01$) the concentration and output of fat in milk (19 and 17%, respectively). Relative to the control and ELFO, EL decreased ($P < 0.05$) milk 10:0-16:0 and odd- and branched-chain FA content and increased 18:0, *cis*-18:1, *trans*- Δ^{13} 18:1 (and their corresponding Δ -9 desaturase products), *trans*-12,*cis*-14 CLA, *cis*-13,*trans*-15 CLA, *cis*-12,*trans*-14 CLA and *trans*-11,*cis*-13 CLA and 18:3n-3 concentrations. ELFO was more effective for enriching ($P < 0.05$) milk *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations (up to 5.4- and 7.1-fold compared with the control) than EL (up to 1.7- and 2.5-fold increases). Furthermore, ELFO resulted in a substantial increase in milk *trans*-10 18:1 concentration (5.4% total FA) with considerable variation between individual animals. Relative to the control and EL, milk fat responses to ELFO were

characterized by increases ($P < 0.05$) in milk *trans*-16:1 ($\Delta 9$ -11), *trans*-18:1 ($\Delta 6$ -11), *trans*-18:2, CLA (*cis*-9,*trans*-11, *trans*-9,*cis*-11, *trans*-8,*trans*-10 and *trans*-7,*trans*-9) and 20- and 22-carbon FA concentrations. Overall, EL resulted in a relatively high *cis*-9-18:1 concentration and an increase in the 18:3 n -3/18:2 n -6 ratio, whereas combining EL and FO resulted in substantial increases in *trans*-FA, marginal enrichment in 20:5 n -3 and 22:6 n -3, and lower 16:0 concentrations changes associated with a decrease in milk fat content. In conclusion, data provide further evidence of differential mammary lipogenic responses to diet in the goat compared with the cow and sheep.

Key words: goat milk, extruded linseed, fish oil, conjugated linoleic acid, *trans* fatty acid

Implications

The present study reports new data on the effect of supplementing diets based on natural grassland hay with extruded linseeds alone or in combination with fish oil, on the intake and production of Alpine goats and associated changes in milk fatty acid composition and milk fat secretion, with specific emphasis on *trans* 18:1, conjugated and non-conjugated 18:2 isomers. Data generated provides further evidence of differential responses to lipid supplements in goats compared with cows and sheep.

Introduction

Nutrition is the major environmental factor regulating milk fat synthesis and fatty acid (FA) composition in ruminants which is an important determinant of the nutritional quality of milk for human consumers. Specific FA including medium-chain saturated FA and certain *trans*-FA are thought to elicit negative effects when consumed in excess, whilst others (*anteiso*-15:0, *cis*-9 18:1, 18:2n-6, *cis*-9,*trans*-11 conjugated linoleic acid (CLA) and 18:3n-3) may have potentially beneficial effects on human health (Shingfield et al, 2008). For these reasons, the opportunities to enhance the concentration of bioactive FA through dietary supplementation with plant oils or seeds rich in n-3PUFA have been explored. Studies in goats (Nudda et al., 2006; Chilliard et al., 2007; Mele et al., 2008; Martínez Marín et al., 2011) and cows (Chilliard et al., 2007) have demonstrated that plant lipid supplements enriched in 18:3n-3 increase milk 18:3n-3 and *cis*-9,*trans*-11 CLA concentrations, with responses being higher in goats than cows (Chilliard and Ferlay, 2004).

It has been suggested that including lipid in the diet as an oilseed rather than oil would limit the extent of ruminal biohydrogenation of PUFA due to seed hulls restricting the access of bacterial lipases to storage triacylglycerol. However, changes in milk 18:3n-3 concentrations to linseed oil or linseed supplements in goats

and cows suggest that 18:3n-3 in whole unprocessed linseeds is more extensively hydrogenated to 18:0 in the rumen compared with 18:3n-3 in free oils (Chilliard et al., 2003, 2007). However, a detailed assessment of dietary supplements of extruded linseeds on milk FA composition has not been documented for dairy goats.

The potential to increase the concentration of 20:5n-3 and 22:6n-3 in milk by including fish oil (FO) to the diet has been examined in cows (Loor et al., 2005a; Shingfield et al., 2006), ewes (Toral et al., 2010), and goats (Kitessa et al., 2001; Gagliostro et al., 2006; Toral et al., accepted). Dietary FO supplements modify rumen biohydrogenation, leading to several-fold enrichment of milk *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations in goats, cows and sheep, with further increases being reported when diets contain plant oils (Gagliostro et al., 2006; Shingfield et al., 2006; Toral et al., 2010). However, the effect of combining FO and 18:3-rich oilseeds such as linseeds on milk production and milk FA composition is not known in goats.

The influence of dietary FO supplements on milk production varies between ruminant species. In cows and ewes, FO typically decreases milk fat content and yield (Chilliard et al., 2001; Loor et al., 2005a; Shingfield et al., 2006; Toral et al., 2010), but reports in goats are limited (Toral et al., accepted).

The present study was conducted to provide a comprehensive evaluation of the effects of dietary supplements of extruded linseeds alone or in combination with FO on performance and milk FA composition in goats with specific emphasis on *trans* FA.

Materials and methods

Animals, management and experimental design

All experimental procedures were approved by the Animal Care Committee of INRA in accordance with the *Use of Vertebrates for Scientific Purposes Act* 1985. Animals were recruited to experiments and allocated to treatment groups according to milk yield, milk fat and protein content, parity, stage of lactation and genotype score at the $\alpha S1$ casein locus. Goats with medium $\alpha S1$ casein content were used since this polymorphism is associated with effects on milk traits and FA composition (Chilliard et al., 2013). Fourteen multiparous (3.6 ± 0.63) Alpine goats in mid-lactation (85 ± 3.3 d in lactation) were offered three experimental diets according to a replicated 3 x 3 Latin Square design with 28 d experimental periods with 4 or 5 animals per group. Fifteen goats were recruited to the experiment, but due to a high milk somatic cell count associated with sharp decrease in milk production at the beginning of the experiment, one animal was withdrawn from the experiment. Each experimental period comprised 21 d adaptation and 7 d interval for sampling and measurements. Goats were housed in a metabolism unit in individual stalls, with continuous access to water and milked at 08.00 and 16.00 h. Diets were formulated to meet energy and protein requirements (INRA, 1989).

Experimental diets

Diets were based on hay prepared from regrowths of natural grassland pasture offered *ad libitum* and a concentrate mixture containing barley and soyabean meal (Table 1). Treatments comprised the basal diet containing no additional lipid (**Control**), 530 g/d of extruded linseeds (extruded mixture of linseed:wheat, 70:30 wt/wt, Union Invivo, Ets Inzo, Chateau-Thierry, France) (**EL**) or 340 g/d of EL and 39 g/d of anchovy FO (SA Daudruy Van Cauwenberghe and Fils, Dunkerque, F-59 640

France) (**ELFO**). EL and ELFO diets were formulated to provide the same amount of FA (Table 1).

Concentrates were fed according to milk yield at the start of experiment in order to represent 50-55% of the DMI. Supplements of EL and FO were mixed with concentrate ingredients before feeding. Concentrate were offered as two equal meals at 08.30 and 16.30 h.

Measurements and sampling

Individual intakes were recorded daily, but only measurements collected during the last week of each experimental period were used for statistical analysis. During each experimental period, representative samples of hay, ingredients of concentrates (barley and soyabean meal), and EL were collected weekly, composited and used to determine the DM content after 48 h at 103°C. Additional subsamples were stored at -20°C for chemical composition and FA analyses. A representative sample of FO was collected weekly, composited and stored at -20°C. Chemical composition of feed ingredients was determined using standard procedures (AOAC, 1997). Milk yields of individual goats were recorded thrice-weekly, while only measurements collected during the last week of each experimental period were analysed statistically. Samples of milk for the measurement of fat, true protein and lactose were collected from each goat over four consecutive milkings starting at 08.00 h on d 21 of each experimental period and treated with preservative (potassium bichromate, Merck, Fontenay-Sous-Bois, France). Milk fat, protein and lactose content were determined by mid infra-red spectroscopy (AOAC, 1997) calibrated using samples of goat milk for which reference measurements had been made. Unpreserved samples of milk were collected over two consecutive milkings starting at 08.00 h on d 22 of each

experimental period, stored at -20°C, composited according to yield and submitted for FA analysis. A sub-sample of unpreserved milk was submitted for the determination of free FA concentrations measured after storage at 4°C for 34 h. For the assay of lipoprotein lipase activity, additional samples of unpreserved milk were collected and stored at -20°C until analysis (Bernard et al., 2005). Live weight of experimental animals was measured at the start and end of each experimental period. Blood samples were collected on d 20 of each experimental period at 07.30 h. Samples from the jugular vein were collected into evacuated collection tubes (Venoject; C.M.L., Nemours, France) containing potassium ethylene diamine tetra-acetic acid. Once collected, blood samples were centrifuged (1500 g for 15 min at 4°C), stored at -20°C and the plasma recovered analysed for insulin and metabolite concentrations (Bernard et al., 2005).

Lipid analysis

Chemical composition of feed ingredients was determined using standard procedures (AOAC, 1997). Fatty acid methyl esters (FAME) of lipid in feed samples were prepared using a 1-step extraction-transesterification (Sukhija and Palmquist, 1988), with 23:0 (Sigma, Saint-Quentin Fallavier, France) as an internal standard. FAME of milk were obtained by based-catalysed transesterification by the incubation of 2 ml of 0.5 M sodium methoxide in methanol and 1 ml of hexane to 100 mg of lyophilised milk at 50°C for 15 min. After cooling, 1 ml of 5% (vol/vol) methanolic hydrochloric acid was added and the reaction mixture was maintained at 50°C for 15 min. Once cool, 3 ml of 6% (wt/vol) of aqueous potassium carbonate and 1.5 ml of hexane were added. Tubes were shaken vigorously, centrifuged at 1570 g for 5 min at 4°C, and the upper organic phase was recovered. Methyl esters were separated and

quantified by gas-liquid chromatography using a gas chromatograph Trace-GC 2000 equipped with a flame-ionization detector (Thermo Finnigan, Les Ullis, France) and 100 m fused silica capillary column (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using hydrogen as the carrier and fuel gas. Total FAME profile was determined in a 0.5 μ L sample at a split ratio of 1:50 using a temperature gradient program (Loor et al., 2005b) and isomers of 18:1 were further resolved in a separate analysis under isothermal conditions (Shingfield et al., 2003). Peaks were routinely identified using authentic FAME standards (GLC#463, Nu-Check Prep Inc, Elysian, MN, USA; iso and ante-iso 13:0, 14:0, 15:0, 16:0, 17:0 and 18:0; Sigma-Aldrich, Saint-Quentin Fallavier, France). Reference butter oil (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors to account for the carbon deficiency in the flame ionization detector response for FAME containing 4 to 10 carbon atoms. Methyl esters not available as commercial standards were identified based on retention time comparisons with milk fat samples for which peaks were identified based on GC-MS analysis of FAME and 4,4-dimethyloxazoline FA derivatives (Shingfield et al., 2006; 2008).

The distribution of CLA isomers in milk fat FAME was determined by HPLC using four silver-impregnated silica columns (ChromSpher 5 lipids, 250 x 4.6 mm; 5 μ m particle size, Varian Ltd., Walton-on-Thames, UK) coupled in series and 0.1 % (vol/vol) acetonitrile in heptane as the mobile phase (Shingfield et al., 2003). Concentrations of CLA isomers were calculated from the proportionate peak area responses determined by HPLC and the sum of concentrations of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA and *cis*-9,*trans*-11 CLA weight percentage determined by GC analysis.

Calculations and statistical analysis

Apparent transfer of 20:5n-3, 22:5n-3, and 22:6n-3 from FO into milk was calculated as: $[\text{g milk FA yield} \times (\text{g FA}/100 \text{ g milk fat} - \text{g FA}/100 \text{ g in control milk fat}) / (\text{DMI} \times \text{g FA intake})] \times 100$.

Experimental data were subjected to Analysis of Variance using the general linear model procedure of Statistical Analysis Systems software package version 8.2 (SAS, SAS Institute, Cary, NC, USA) with a model that included the fixed effects of period and treatment and random effects of goat. Least square means \pm SEM are reported and treatment effects were declared significant at $P < 0.05$ and considered a trend towards significance at $P < 0.10$.

Pearson correlation coefficients (r) were generated for the association between individual FA in milk fat and between the abundance of specific milk FA with milk fat content using the CORR procedure of SAS.

Results

Diet composition

Natural grassland hay was of high quality in term of nutritional value and had the following composition (g/kg DM, unless otherwise stated): DM (g/kg fresh weight), 855; organic matter, 907; crude protein, 98; acid-detergent fibre, 281; neutral-detergent fibre, 524; FA content, 17. Concentrations of organic matter and starch were higher for the control than EL and ELFO, whereas acid-detergent fibre, neutral-detergent fibre, crude protein, diethyl ether extract, and total FA content were lower (Table 1).

The content of organic matter, neutral-detergent fibre, crude protein, starch, diethyl ether extract, and total FA were similar for EL and ELFO, other than a marginally lower amount (-3%) of acid-detergent fibre for ELFO than EL. By design, the EL and ELFO increased the concentration of specific FA in the diet (Table 1).

Dietary forage:concentrate ratio of the diet (on a DM basis) averaged 52:48, 56:44 and 55:45 for the control, EL and ELFO, respectively.

Animal performance

EL diet supplied the highest amounts of 18:0, *cis*-9 18:1, 18:2n-6 and 18:3n-3 (5.6, 29.9, 28.6, 74.4 g per d, respectively), whereas the ELFO treatment was a source of 20:5n-3 and 22:6n-3 (3.1 and 2.3 g per d, respectively). In ELFO and EL, *cis*-9 18:1, 18:2n-6 and 18:3n-3 were the major FA provided by the diets (26, 24 and 51 g per d, respectively for ELFO, and 30, 29 and 74 g per d, respectively for EL). 18:2n-6 was the major FA provided by the control diet (13.5 g per d), 18:3n-3 was the major FA provided by the 2 lipid supplemented diets and was 31% lower in ELFO diet compared to EL (Table 2).

Compared with the control and EL, ELFO lowered ($P = 0.02$) DM intake (Table 2). Treatments had no effect ($P > 0.05$) on the yields of milk, milk protein or lactose. However, compared with the control and ELFO, EL increased ($P < 0.01$) milk fat yield and milk fat and protein content, whereas these parameters did not differ ($P > 0.05$) between the control and ELFO (Table 2). Both EL and ELFO enhanced ($P < 0.001$) lactose concentration relative to the control (Table 2), with the increase being greater for EL than ELFO treatment.

Energy and protein balances (INRA, 1989) were positive for all the dietary treatments. Energy balance was similar among dietary treatments, whereas protein balance was slightly lower for the control compared with EL and ELFO (Table 2).

Plasma metabolite concentrations and milk lipolytic activity

Dietary treatments had no effect ($P > 0.05$) on glucose concentrations while ELFO tended ($P = 0.09$) to decrease plasma insulin concentration compared with the control (Table 3).

Relative to the control, EL and ELFO lowered ($P < 0.001$) plasma acetate and 3-hydroxybutyrate concentrations, with greater decreases ($P < 0.05$) observed for EL than ELFO. Compared with the control and ELFO, EL increased ($P < 0.001$) plasma NEFA concentrations. Even though lipid supplements had no effect ($P > 0.05$) on milk LPL activity, EL decreased ($P < 0.05$) free FA concentration after storage of milk for 34 h at 4°C (Table 3).

Milk fatty acid composition

Both EL and ELFO altered milk FA composition compared the control, changes characterized by decreases ($P < 0.05$) in 8:0 to 16:0, *cis*-9 10:1, *cis*-9 14:1 and *cis*-9 17:1, branched chain FA and an increase in total 18 carbon FA concentration (Table 4).

Relative to the control, only EL increased ($P < 0.05$) milk of 18:0, *cis*-15 18:1 and 18:2n-6 concentrations. Both EL and ELFO enhanced ($P < 0.05$) milk 18:3n-3 concentration, with the response being higher ($P < 0.05$) for EL. Compared with the control and EL, ELFO enriched ($P < 0.05$) milk *cis*-11 20:1, 20:4n-3, 20:5n-3, 22:4n-3, 22:5n-3 and 22:6n-3 concentrations (Table 4).

Dietary supplements of EL alone or in combination with FO elevated ($P < 0.001$) milk *trans*-18:1 concentration (Table 3), with the increases compared the control being higher for ELFO (+698%) than EL (+238%).

Both EL and ELFO enhanced ($P < 0.05$) milk *trans*-4-9 and 13 + 14 18:1 concentrations, while increases ($P < 0.01$) in *trans*-10 18:1 and *trans*-11 18:1 were confined to ELFO diet resulting in concentrations 16- and 7-fold higher than the control diet, respectively (Table 5). Overall, *trans*-10 and *trans*-11 accounted for 39.5% and 39.3% of total milk *trans*-18:1 content on the ELFO treatment.

Concentrations of total *cis*-18:1 increased ($P < 0.001$) in response to EL (+79%), with enrichment of *cis*-9 18:1 accounting for 90 % of the total increase in *cis*-18:1. Both EL and ELFO enhanced *cis*-13 18:1 and *cis*-14 18:1 content, whereas ELFO resulted in *cis*-11 18:1 enrichment (Table 5).

Dietary lipid supplements altered the relative abundance of non-conjugated 18:2 isomers (Table 6). Compared with the control, EL and ELFO increased ($P < 0.001$) milk fat *cis*-9, *trans*-12 18:2 and *cis*-9,*trans*-13 18:2 concentrations and ELFO enhanced ($P < 0.001$) the abundance of *trans*-9,*trans*-12 18:2 and *trans*-11,*cis*-15 18:2 (Table 6).

Apparent transfer of 20:5n-3 and 22:6n-3 from the diet into milk for the ELFO treatment were marginal (2.9 and 3.7% respectively) and much lower than for 22:5n-3 (10.1%; data not presented).

Compared with the control and EL, ELFO increased ($P < 0.001$) total CLA concentrations (mean responses +175 and +414%, respectively) with *cis*-9,*trans*-11 CLA being the major isomer. Most CLA isomers detected in milk were enhanced ($P < 0.001$) by ELFO, and to a lower extent by EL. Both EL and ELFO elevated ($P <$

0.001) *trans*-7,*cis*-9 CLA, *cis*-11,*trans*-13 CLA, *trans*-11,*cis*-13 CLA, *trans*-12,*trans*-14 CLA and *trans*-13,*trans*-15 CLA.

Compared with the control, EL resulted in the specific enrichment ($P = 0.001$) in order of the relative abundance, *trans*-12,*cis*-14 CLA, *trans*-11,*trans*-13 CLA and *cis*-12,*trans*-14 CLA (Table 6). Relative to EL, ELFO increased ($P = 0.001$) in order of relative abundance, *cis*-9,*trans*-11 CLA, *trans*-9,*cis*-11 CLA, *trans*-9,*trans*-11 CLA, *trans*-8,*trans*-10 CLA, *trans*-10,*trans*-12 CLA, *trans*-7,*trans*-9 CLA and *trans*-6,*trans*-8 CLA.

Discussion

Several studies have examined the effect of dietary plant lipid supplements on milk production and milk FA composition in the dairy goat (Chilliard et al., 2007; Mele et al., 2008; Bernard et al., 2009), but few have investigated the interaction of plant oils or oilseeds with FO in this species (Toral et al., accepted). The present study provided a comprehensive assessment of including extruded linseed alone or in combination with FO on the performance and milk FA profile of goats fed diets based on grass hay.

Animal performance

Feed consumption was decreased by 6% in goats in response to ELFO as typically observed in cows with FO addition to the diets (Whitlock et al., 2002).

Milk production and composition responses to extruded linseeds were consistent with previous studies (Chilliard and Ferlay, 2004; Chilliard et al. 2007, 2013) supporting the view that oilseeds and plant oils typically have no effect on milk yield, increase milk fat concentration and secretion, but have variable effects on milk protein

concentration in goats. In contrast, supplements of EL have been shown to lower milk fat content and yield in lactating cows, with the decrease being more pronounced for diets based on maize silage than grass hay (Ferlay et al., 2013). Inclusion of FO in the diet counteracted the positive effect of EL on milk fat synthesis, highlighting that FO may modify milk fat responses to oilseed supplements in goats. Due to the lack of data, indirect comparison of responses to EL when FO is included in diet between ruminant species is not possible.

A recent study reported that supplementing the diet with a 31:69% (wt/wt) mixture of FO and linseed oil (5.8 g/kg DM) increased milk fat secretion and content in goats (Toral et al., accepted). The reason for the differences between studies may be due to the form of supplementary lipid, and basal diet composition, in particular the nature and amount of starch (128 g/kg DM from corn (Toral et al., accepted) vs 169 g /kg DM from barley present study) which is a major determinant of milk fat responses to plant lipids in lactating cows (Shingfield et al, 2010). However, differences in dietary starch content have been demonstrated to have no effect on milk fat yield and content responses to FO in goats (Toral et al., accepted) or to a mixture of FO and sunflower-seed oil in cows (Shingfield et al., 2005).

Milk fatty acid composition

Response to extruded linseeds

The impact of dietary EL supplementation (equivalent to an additional 52 g oil/kg DM) on the concentrations of the major FA in milk is consistent with earlier studies in lactating goats and cows (Chilliard et al., 2007; 2013), characterized by decreases in medium-chain saturated FA and increases in 18:0, *cis*-9 18:1 and 18:3n-3. The reduction in medium-chain saturated FA to EL, may at least in part, be related to

lower plasma concentrations of acetate and 3-hydroxybutyrate that serve as precursors for *de novo* FA synthesis in the mammary gland. Supplements of EL specifically increased *cis*-9 18:1, *cis*-12 18:1, *cis*-15 18:1 and *cis*-9,*trans*-12 CLA. Indirect comparisons of changes in milk fat composition for cows fed similar diets (Lerch et al., 2012a,b; Ferlay et al., 2013) indicate the increase in milk *cis*-9 18:1 and 18:0 content to EL supplements can be expected to be higher in goats, consistent with the findings of earlier studies (Chilliard et al., 2007; 2013). Moreover, milk fat content was positively correlated with milk 18:0 concentration (Figure 1 and Table 7; $r=0.61$), providing additional support to the hypothesis that this substrate is a major factor regulating mammary lipogenesis in goats (Chilliard and Ferlay, 2004).

The EL treatment (216 g EL/kg DM) increased milk *trans*-11 18:1 (+147%) and *cis*-9,*trans*-11 CLA (+69%) concentrations, in line with other data in goats with extruded linseed (Nudda et al., 2006; Chilliard et al., 2007), but in lower magnitude than recently observed in response to 170 g EL/kg DM in goats offered diets containing less starch (and using the same extrusion method to extrude linseeds) (Chilliard et al., 2013), differences that may be associated with the higher starch content of the basal diet in the present study (hay-barley based diet). However, the enrichment of milk *trans*-11 18:1 and *cis*-9,*trans*-11 CLA on the EL treatment was higher than the increases to 50 g EL/kg DM reported for cows fed a similar basal diet (50% hay containing 114 g starch/kg DM; Ferlay et al., 2013). Conversely, EL had no influence on milk *trans*-10 18:1 and *trans*-10,*cis*-12 CLA concentrations, the abundance of which was found to increase in cows (Ferlay et al., 2013). Differential responses between ruminant species may be associated with a greater stability of the ruminal *trans*-11 biohydrogenation pathway in the goat compared with the cow, in which a shift to the *trans*-10 pathway is more frequently observed (Shingfield et al., 2010).

As expected, milk 18:3n-3 concentration and the $\sum n-3/\sum n-6$ ratio were markedly increased on the EL treatment (Table 4). An enrichment of 18:3n-3 in milk of up to 2.19 g/100 g on a diet supplying 30.6 g 18:3 n-3/kg DM is higher than the abundance in milk of 1.53 g/100 g from cows fed a similar diet providing 27.2 g 18:3n-3 /kg DM (Ferlay et al., 2013). This observation is in line with previous reports of a greater increase in milk 18:3n-3 concentration to linseed based supplements in the goat compared with the cow (Chilliard et al., 2007) which may be related to less extensive biohydrogenation of dietary PUFA in the rumen of goats than cows. Other hypothesis such as differences among goats and cows of 18:3n-3 partitioning among tissues and/or mammary extraction from circulating 18:3n-3 would also merit to be investigated.

Response to extruded linseeds and fish oil

The ELFO treatment increased the $\sum n-3/\sum n-6$ ratio in milk due to 18:3n-3 and long-chain n-3 PUFA enrichment (Table 4). Given the relatively high concentration of 20:5n-3, 22:5n-3 and 22:6n-3 on the control and EL treatment and for non-supplemented diets in earlier studies with goats (Toral et al., accepted), the apparent transfer of long-chain n-3PUFA (20:5n-3, 22:5n-3 and 22:6n-3) were calculated taking into account their secretion on the control. The efficiency of transfer (3.7-10.1%) was in the same range reported for goats fed FO alone or as a mixture with plant oils (from 1.4 to 3.4% for 20:5n-3 and 22:6n-3; Toral et al., submitted). Transfer of 22:5n-3 was higher (~ 10%) as has been reported previously in goats (Toral et al., accepted), cows (Lor et al., 2005a) and ewes (Toral et al., 2010). Part of the higher transfer efficiency may be explained by a lower apparent disappearance of 22:5n-3 in the rumen (Lee et al., 2008; Shingfield et al., 2012) and higher extraction and uptake

of 22:5n-3 across the mammary gland (Offer et al., 1999; Chilliard et al., 2000; Loores et al., 2005a).

Results indicate that supplements of FO and EL is more effective for increasing milk *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations compared with EL alone which is in agreement with previous observations on the interaction between FO and plant oils in cows and the inhibitory effect of certain FA in FO on the reduction of *trans* 18:1 isomers to 18:0 in the rumen (AbuGhazaleh et al., 2003; Shingfield et al., 2006; Chilliard et al., 2007).

The ELFO caused greater increases in milk *trans*-10 18:1 than EL resulting in an equal abundance of *trans*-10 18:1 and *trans*-11 18:1. A milk *trans*-10 18:1 concentration of 5.4 g/100 g FA is much higher than 1.04 g/100 g FA in a previous experiment with goats fed FO and linseed oils (Toral et al., accepted). Differences between studies may be partly due to differences in the form of linseed lipid and dietary starch content. However, increases in the starch content of diets based on lucerne hay had no effect on milk *trans*-10 18:1 in goats offered FO supplements (Toral et al., accepted), suggesting that interactions between the type of linseed supplement and basal diet, in particular the nature of starch (barley present study vs corn and/or barley Toral et al., (accepted)), may, at least in part, explain these differences. Enrichment of *trans*-10 18:1 on the ELFO treatment is notable given that the stability of the *trans*-11 ruminal biohydrogenation pathway is generally greater in the goat than cow and concentrations of *trans*-10 18:1 are typically lower than 3.5 g/100 g FA in milk from goats fed high starch diets supplemented with plant oils (Chilliard et al., 2007; Bernard et al., 2009).

Milk *trans*-10 18:1 concentration (mean 5.4% SD 6.96) varied considerably between individual animals. Enrichment in milk for four goats ranged between 13 and 19 g/100

g FA and was not associated with a decrease in milk fat content. In the goat, increases in milk *trans*-10 18:1 concentrations are associated with marginal increases in milk fat yield, whereas the reverse is true in cows (Shingfield et al., 2010). Furthermore, the lack of an association between that milk *trans*-10 18:1 concentration and milk fat synthesis in the present study suggests that other biohydrogenation intermediates or other factors may account for the suppression of increases in milk fat yield to EL when FO is included in the diet. Examination of the association between milk fat content and milk FA composition highlighted a negative association with milk *cis*-11 16:1, *cis*-11 18:1, *cis*-11 20:1, *trans*-8,*trans* 10 CLA, and *trans*-7,*trans*-9 CLA concentrations (Figure 1; Table 7). A similar relationship between milk fat and milk *cis*-11 18:1 concentration has been reported for cows fed supplements of FO and sunflower oil (Shingfield et al., 2006). However, the control diet also contained *cis*-11 18:1, while *cis*-11 16:1 and *cis*-11 20:1 supplied exclusively on the ELFO treatment originated from FO (Table 4). Previous study suggests that the appearance of *cis*-11 16:1 and *cis*-11 20:1 in milk is related to ruminal escape rather than formation during biohydrogenation of FA in EL or FO (Kairenius et al., 2011). Studies in bovine adipocytes (Burns et al., 2006) have demonstrated that *cis*-11 18:1 can be synthesized endogenously by the elongation of *cis*-9 16:1. It is therefore possible that *cis*-11 16:1 and *cis*-11 20:1 may originate from the elongation of *cis*-9 14:1 and *cis*-9 18:1, respectively, in adipose which during mobilization of body tissue and uptake across the mammary gland could be made available for milk fat synthesis. In vitro, *cis*-11 18:1 has been shown to lower lipogenesis and FASN gene expression in bovine adipocytes (Burns et al., 2006), highlighting that this FA may act as an inhibitor of lipogenesis at least in adipose. Even though the mode of action of this FA on lipogenesis in adipocyte has not been

elucidated, the possibility that *cis*-11 18:1 inhibits mammary lipogenesis cannot be excluded. However, post-ruminal infusion studies of a mixture of 18:1 (30 g/d) isomers containing 12.5% of *cis*-11 18:1 were found to have no effect on milk fat synthesis in cows (Shingfield et al., 2007) suggesting a neutral effect of this isomer at least in cows.

A negative relationship between milk fat content and milk *cis*-11 16:1 or *cis*-11 20:1 concentrations may be related to a co-dependence between these parameters rather than a direct effect on lipogenesis. A negative association was also observed for *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA but there is no direct evidence on the role of these isomers in the regulation of mammary lipogenesis in ruminants.

In cows, relatively few biohydrogenation intermediates are known (*trans*-10,*cis*-12 CLA) or putative (*trans*-9,*cis*-11 CLA, *cis*-10,*trans*-12 CLA) inhibitory effects on mammary lipogenesis (Shingfield et al., 2010), and it is possible that others may be active in the goat, including *trans*-7,*trans*-9 CLA and *trans*-8, *trans*-10 CLA. The ELFO treatment increased *trans*-9,*cis*-11 CLA concentrations 14.5-fold (0.058 g/100 g FA). In earlier studies, *trans*-9,*cis*-11 CLA was not detected in milk from goats fed hay- or maize silage-based diet supplemented with plant oils, whereas *trans*-9,*trans*-11 CLA was increased (Bernard et al., 2009). An increase in milk *trans*-9,*cis*-11 CLA content was confined to the same individuals for which concentrations of *trans*-10 18:1 were elevated which explains the close association between these FA in milk (Table 7). Similarly, a close relationship between *trans*-9,*cis*-11 CLA and *trans*-10 18:1 concentrations have been reported in milk of cows (Shingfield et al., 2006) and sheep (Toral et al., 2010) fed with a mixture of sunflower oil and marine oils or from cows fed diets containing oilseeds (Lerch et al., 2012a,b). Collectively, these observations suggest significant variability in the functioning and diversity of the

rumen microbiome and adaptations to changes in diet composition between ruminant species that merits further investigation.

Furthermore, milk fat secretion and FA composition responses to the ELFO treatment demonstrated considerable between-animal variation in the synthesis and secretion of *trans*-9,*cis*-11 CLA, and for *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA that may also have an influence on milk fat content and yield in goats. These changes were also accompanied by the prevention of an increase in 18:0 supply which may have suppressed the increase in milk fat synthesis observed on the EL treatment. Earlier studies have provided evidence to suggest that a shortage of 18:0 for endogenous *cis*-9 18:1 synthesis may explain or contribute to FO induced milk fat depression in cows (Lor et al., 2005a; Shingfield et al, 2006) and ewes (Toral et al., 2010) due to compromised milk fat fluidity. Overall, a combination of all these alterations may have contributed to FO suppressing the positive effect of EL on milk fat content.

Milk lipolysis

Dietary supplements of EL had an adverse effect on post-milking milk free FA concentrations consistent with lower levels of spontaneous lipolysis in milk from goats supplemented with 18:3n-3-rich lipids (Chilliard et al., 2003, 2013; Eknæs et al., 2009). Such changes may influence the sensory quality of milk by reducing the development of goat flavour (Chilliard et al., 2003). A numerical, but non-significant difference compared with the control was also detected when FO was fed with EL (Table 3). These observations are in line with previous reports in goats fed a combination of plant oils and FO or FO alone (Toral et al., accepted), which suggests that FO has limited influence or may compensate for the effects of plant lipid supplements on milk fat lipolysis.

Conclusions

Supplementing hay based diets rich in extruded linseed increases milk fat content and yield in goats and alters milk FA composition, characterised by decreases in SFA and increases in 18:0, *cis* 18:1, *trans* 18:1, CLA and 18:3n-3. A strong positive relationship between milk fat content and milk 18:0 concentration reinforce the hypothesis that the supply of 18:0 is involved in the regulation of mammary lipid secretion in goats. In contrast to cows, supplements of FO and EL had no influence on milk fat content and yield in goats. However, when compared with supplements of EL alone, FO prevented the increase in milk fat synthesis to EL and induced larger increases in *trans* 18:1 and CLA isomers in milk fat. Data suggest a specific effect of a mixture of FO and EL on ruminal accumulation and secretion of *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA and *trans*-9,*cis*-11 CLA in milk, isomers that along with a decrease in 18:0 supply may explain the adverse effects on milk fat synthesis. Direct inter-species comparisons are required to define differential responses to dietary FO supplements and their interaction with plant lipids. A more complete understanding of the diversity and functioning of the rumen microbiome may offer an explanation for species specific differences in lipid digestion and metabolism and diet-induced changes in milk fat composition.

Acknowledgments

The present study was supported by Apis-Gene and the Agence Nationale de la Recherche (ANR, France; ANR-GANI-014, coordinated by Patrice Martin). Authors gratefully acknowledge the staff of Unité Expérimentale des Ruminants de Theix (INRA, France) for the diligent care of the experimental animals and P. Capitan, E.

536 Tixier, D. Bany and M. Tourret from UMR 1213 Herbivores (INRA, France) for
537 assistance in the management of the animals, sampling and laboratory analysis, and
538 L. Ventto and P. Kairenius (MTT, Finland) for technical support during the analysis of
539 milk fatty acid composition.

540

References

- AbuGhazaleh AA, Schingoethe DJ, Hippen AR and Kalscheur KF 2003. Conjugated linoleic acid and vaccenic acid in rumen, plasma, and milk of cows fed fish oil and fats differing in saturation of 18 carbon fatty acids. *Journal of Dairy Science* 86, 3648-3660.
- Association of Official Analytical Chemists (1997) *Official Methods of Analysis*, 16th ed. Gaithersburg, MD: AOAC International.
- Bernard L, Shingfield KJ, Rouel J, Ferlay A and Chilliard Y 2009. Effect of plant oils in the diet on performance and milk fatty acid composition in goats fed diets based on grass hay or maize silage. *British Journal of Nutrition* 101, 213-224.
- Bernard L, Rouel J, Leroux C, Ferlay A, Faulconnier Y, Legrand P and Chilliard Y 2005. Mammary lipid metabolism and milk fatty acid secretion in alpine goats fed vegetable lipids. *Journal of Dairy Science* 88, 1478-1489.
- Burns TA, Kadegowda AKG, Duckett SK, Pratt SL and Jenkins TC 2012. Palmitoleic (16:1 cis-9) and cis-vaccenic (18:1 cis-11) acid alter lipogenesis in bovine adipocyte cultures. *Lipids* 47, 1143-1153.
- Chilliard Y and Ferlay A 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reproduction Nutrition Development* 44, 467-492.
- Chilliard Y, Ferlay A and Doreau M 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science* 70, 31-48.
- Chilliard Y, Ferlay A, Mansbridge RM and Doreau M 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Annales De Zootechnie* 49, 181-205.
- Chilliard Y, Ferlay A, Rouel J and Lamberet G 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science* 86, 1751-1770.
- Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J and Doreau M 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology* 109, 828-855.

571 Chilliard Y, Rouel J and Guillouet P 2013. Goat alpha-s1 casein genotype interacts with the
572 effect of extruded linseed feeding on milk fat yield, fatty acid composition and post-milking
573 lipolysis. *Animal Feed Science and Technology* 185, 140-149.

574 Eknæs M, Havrevoll Ø, Volden H and Hove K 2009. Fat content, fatty acid profile and off-
575 flavours in goats milk-Effects of feed concentrates with different fat sources during the
576 grazing season. *Animal Feed Science and Technology* 152, 112-122.

577 Ferlay A, Doreau M, Martin C and Chilliard Y 2013. Effects of incremental amounts of
578 extruded linseed on the milk fatty acid composition of dairy cows receiving hay or corn silage.
579 *Journal of Dairy Science* 96, 6577-6595.

580 Gagliostro GA, Rodríguez A, Pellegrini PA, Gatti P, Muset G, Castañeda RA, Colombo D and
581 Chilliard Y 2006. Effects of fish oil or sunflower plus fish oil supplementation on conjugated
582 linoleic acid (CLA) and omega 3 fatty acids in goat milk. *Revista Argentina de Produccion*
583 *Animal* 26, 71-87.

584 INRA 1989. In: Jarrige, R. (Ed.), *Recommended allowances and feed tables*. John Libbey
585 Eurotext, London, England, Paris, France. .

586 Kairenius P, Toivonen V and Shingfield KJ 2011. Identification and ruminal outflow of long-
587 chain fatty acid biohydrogenation intermediates in cows fed diets containing fish oil. *Lipids*
588 46, 587-606.

589 Kitessa SM, Gulati SK, Ashes JR, Fleck E, Scott TW and Nichols PD 2001. Utilisation of fish
590 oil in ruminants - II. Transfer of fish oil fatty acids into goats' milk. *Animal Feed Science and*
591 *Technology* 89, 201-208.

592 Lee MRF, Shingfield KJ, Tweed JKS, Toivonen V, Huws SA and Scollan ND 2008. Effect of
593 fish oil on ruminal biohydrogenation of C18 unsaturated fatty acids in steers fed grass or red
594 clover silages. *Animal* 2, 1859-1869.

595 Lerch S, Shingfield KJ, Ferlay A, Vanhatalo A and Chilliard Y 2012a. Rapeseed or linseed in
596 grass-based diets: Effects on conjugated linoleic and conjugated linolenic acid isomers in
597 milk fat from Holstein cows over 2 consecutive lactations. *Journal of Dairy Science* 95, 7269-
598 7287.

599 Lerch S, Ferlay A, Shingfield KJ, Martin B, Pomiès D and Chilliard Y 2012b. Rapeseed or
600 linseed supplements in grass-based diets: Effects on milk fatty acid composition of Holstein
601 cows over two consecutive lactations. *Journal of Dairy Science* 95, 5221-5241.

602 Loor JJ, Doreau M, Chardigny JM, Ollier A, Sebedio JL and Chilliard Y 2005a. Effects of
603 ruminal or duodenal supply of fish oil on milk fat secretion and profiles of trans-fatty acids
604 and conjugated linoleic acid isomers in dairy cows fed maize silage. *Animal Feed Science*
605 *and Technology* 119, 227-246.

606 Loor JJ, Ferlay A, Ollier A and Chilliard Y 2005b. Relationship among trans and conjugated
607 fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. *Journal of*
608 *Dairy Science* 88, 726-740.

609 Martínez Marín AL, Gómez-Cortés P, Gómez Castro AG, Juárez M, Pérez Alba LM, Pérez
610 Hernández M and de la Fuente MA 2011. Animal performance and milk fatty acid profile of
611 dairy goats fed diets with different unsaturated plant oils. *Journal of Dairy Science* 94, 5359–
612 5368.

613 Mele M, Buccioni A, Serra A, Antongiovanni M and Secchiari P 2008. Lipids of goat's milk:
614 origin, composition and sources of variation. In *Dairy goats feeding and nutrition*, Eds A.
615 Cannas and G. Pulina, CAB International, 47-70.

616 Nudda A, Battacone G, Usai MG, Fancellu S and Pulina G 2006. Supplementation with
617 extruded linseed cake affects concentrations of conjugated linoleic acid and vaccenic acid in
618 goat milk. *Journal of Dairy Science* 89, 277-282.

619 Offer NW, Marsden M, Dixon J, Speake BK and Thacker FE 1999. Effect of dietary fat
620 supplements on levels of n-3 poly-unsaturated fatty acids, trans acids and conjugated linoleic
621 acid in bovine milk. *Animal Science* 69, 613-625.

622 Shingfield KJ, Ahvenjärvi S, Toivonen V, Äröla A, Nurmela KVV, Huhtanen P and Griinari JM
623 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in
624 cows. *Animal Science* 77, 165-179.

625 Shingfield KJ, Ahvenjarvi S, Toivonen V, Vanhatalo A and Huhtanen P 2007. Transfer of
626 absorbed cis-9, trans-11 conjugated linoleic acid into milk is biologically more efficient than
627 endogenous synthesis from absorbed vaccenic acid in lactating cows. *Journal of Nutrition*
628 137, 1154-1160.

629 Shingfield KJ, Bernard L, Leroux C and Chilliard Y 2010. Role of trans fatty acids in the
630 nutritional regulation of mammary lipogenesis in ruminants. *Animal* 4, 1140-1166.

- Shingfield KJ, Reynolds CK, Hervás G, Griinari JM, Grandison AS and Beever DE 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *Journal of Dairy Science* 89, 714-732.
- Shingfield KJ, Ahvenjarvi S, Toivonen V, Vanhatalo A, Huhtanen P and Griinari JM 2008. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. *British Journal of Nutrition* 99, 971-983.
- Shingfield KJ, Reynolds CK, Lupoli B, Toivonen V, Yurawecz MP, Delmonte P, Griinari JM, Grandison AS and Beever DE 2005. Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. *Animal Science* 80, 225-238.
- Shingfield KJ, Kairenius P, Ärölä A, Paillard D, Muetzel S, Ahvenjärvi S, Vanhatalo A, Huhtanen P, Toivonen V, Griinari JM and Wallace RJ 2012. Dietary fish oil supplements modify ruminal biohydrogenation, alter the flow of fatty acids at the omasum, and induce changes in the ruminal butyrivibrio population in lactating cows. *Journal of Nutrition* 142, 1437-1448.
- Sukhija PS and Palmquist DL 1988. Rapid method for determination of total fatty-acid content and composition of feedstuffs and feces. *Journal of agricultural and food chemistry* 36, 1202-1206.
- Toral PG, Rouel J, Bernard L and Chilliard Y 2014. Interaction between fish oil and plant oils or starchy concentrates in the diet: Effects on dairy performance and milk fatty acid composition in goats. *Animal Feed Science and Technology*, accepted.
- Toral PG, Frutos P, Hervás G, Gómez-Cortés P, Juárez M and de la Fuente MA 2010. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. *Journal of Dairy Science* 93, 1604-1615.
- Whitlock LA, Schingoethe DJ, Hippen AR, Kalscheur KF, Baer RJ, Ramaswamy N and Kasperson KM 2002. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. *Journal of Dairy Science* 85, 234-243.

Figure caption

Figure 1 Relationships between milk fat content (g/kg) and concentrations of *cis*-11 16:1, *cis*-11 18:1, *cis*-11 20:1, 18:0, *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA in milk (g/100g of fatty acids) from goats fed grass hay based diets supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO). Relationships derived using 42 measurements made for 14 animals.

670
671

Table 1 *Formulation and chemical composition of experimental diets*

Ingredient (g/kg dry matter)	Treatment		
	Control	EL	ELFO
Natural grassland hay	518	563	558
Barley	449	208	247
Soya bean meal	32	12	30
Extruded linseeds ¹	0	216	147
Fish oil ²	0	0	17
Mineral and vitamin premix ³	4	4	4
Chemical composition (g/kg dry matter)			
Organic matter	936	932	933
Crude protein	116	129	129
Neutral detergent fibre	366	384	376
Acid detergent fibre	171	194	188
Starch	247	159	169
Diethyl ether extract	17	69	69
14:0	0.09	0.11	1.74
15:0	0.03	0.04	0.15
16:0	3.65	6.14	8.54
<i>cis</i> -9 16:1	0.04	0.09	1.58
<i>cis</i> -11 16:1	0.00	0.00	0.10
17:0	0.03	0.12	0.16
18:0	0.33	2.30	2.20
<i>cis</i> -9 18:1	2.17	12.32	11.64
<i>cis</i> -11 18:1	0.14	0.60	0.96
18:2n-6	5.46	11.79	10.39
18:3n-3	2.16	30.64	22.36
20:0	0.09	0.14	0.16
<i>cis</i> -11 20:1	0.00	0.00	0.46
22:0	0.10	0.16	0.14
20:5n-3	0.00	0.00	1.37
24:0	0.09	0.13	0.13
22:5n-3	0.09	0.11	0.38
22:6n-3	0.00	0.00	1.02
Other fatty acids	1.06	1.63	3.96
Σ Fatty acids	16	66	67
Energy (MJ/kg dry matter) ⁴	6.62	6.90	6.98
Protein (g PDI/ kg dry matter) ⁵	76	87	86

672 ¹Extruded linseeds contained (g/kg DM) 16:0 (16.9), 18:0 (9.6), *cis*-9 18:1 (50.5), 18:2n-6 (40.2),
673 18:3n-3 (131.8) and total fatty acids (256).

674 ²Fish oil contained (g/kg FA) 14:0 (98.9), 16:0 (195.8), *cis*-9 16:1 (90.8), *cis*-11 16:1 (2.4), 18:0 (29.9),
675 *cis*-9 18:1 (145.8), *cis*-11 18:1 (30.0), 18:2n-6 (38.2), 18:3n-3 (16.6), *cis*-11 20:1 (27.6), 20:5n-3 (82.7),
676 22:5n-3 (16.6), 22:6n-3 (61.8) and total fatty acids (992).
677 ³Mineral-vitamin premix declared as containing (g/kg): Ca (240), P (60), Mg (50), Na (15), Zn, (7), Mn
678 (6), α-dl-tocopherol (0.3), retinol (0.2) and cholecalciferol (0.002) (Usine d'Ussel, Murat, France).
679 ⁴Net energy for lactation calculated according to INRA (1989).
680 ⁵Digestible protein at the intestine calculated according to INRA (1989).
681

Table 2 Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on dry matter intake, fatty acid intake, milk yield and milk composition in lactating goats

	Treatment ¹			SEM ²	P
	Control	EL	ELFO		
Dry matter (Kg/day)	2.47 ^b	2.45 ^b	2.31 ^a	0.041	0.013
Fatty acid intake (g/day)					
14:0	0.22 ^b	0.27 ^b	3.95 ^a	0.038	< 0.001
16:0	9.04 ^c	14.94 ^b	19.47 ^a	0.170	< 0.001
<i>cis</i> -9 16:1	0.11 ^c	0.21 ^b	3.58 ^a	0.035	< 0.001
18:0	0.82 ^c	5.57 ^a	5.00 ^b	0.069	< 0.001
<i>cis</i> -9 18:1	5.38 ^c	29.91 ^a	26.45 ^b	0.361	< 0.001
<i>cis</i> -11 18:1	0.36 ^c	1.45 ^b	2.18 ^a	0.021	< 0.001
18:2n-6	13.52 ^c	28.64 ^a	23.65 ^b	0.265	< 0.001
18:3n-3	5.36 ^c	74.38 ^a	50.88 ^b	0.904	< 0.001
<i>cis</i> -11 20:1	0.00 ^b	0.00 ^b	1.04 ^a	0.011	< 0.001
20:5n-3	0.00 ^b	0.00 ^b	3.11 ^a	0.031	< 0.001
22:5n-3	0.22 ^c	0.26 ^b	0.86 ^a	0.009	< 0.001
22:6n-3	0.00 ^b	0.00 ^b	2.32 ^a	0.023	< 0.001
Yield (g/day)					< 0.001
Milk	3019	2895	2938	51	0.111
Fat	91 ^a	100 ^b	83 ^a	3.095	0.008
Protein	89	87	84	1.502	0.225
Lactose	144	148	146	2.558	0.390
Concentration (g/kg)					
Fat	30.0 ^a	34.6 ^b	27.9 ^a	1.053	< 0.001
Protein	29.5 ^a	30.8 ^b	28.9 ^a	0.302	< 0.001
Lactose	47.4 ^a	51.1 ^c	49.6 ^b	0.276	< 0.001
Energy balance ³ (MJ/d)	2.91	3.08	3.03	0.285	0.831
Protein balance ⁴ (g PDI/d)	11 ^a	32 ^b	29 ^b	3.5	< 0.001

¹Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

²SEM for *n*=14

³Net energy for lactation, balance calculated according to INRA (1989).

⁴PDI = digestible protein at the intestine, balance calculated according to INRA (1989).

^{a, b, c}Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

Table 3 Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on plasma insulin and metabolite concentrations, milk lipoprotein lipase activity and free fatty acid concentrations in lactating goats.

Metabolite	Treatment ¹			SEM ²	P
	Control	EL	ELFO		
Glucose, (mM)	3.39	3.25	3.20	0.166	0.160
NEFA (mM)	0.201 ^b	0.477 ^a	0.203 ^b	0.046	<0.001
Acetate (mM)	0.389 ^a	0.187 ^c	0.260 ^b	0.022	<0.001
3-Hydroxybutyrate (mM)	0.298 ^a	0.126 ^c	0.239 ^b	0.020	<0.001
Insulin (μIU/ml)	17.56	16.20	14.19	1.043	0.087
Lipoprotein lipase(nmol/min per ml)	244.9	231.7	242.7	8.544	0.526
Free fatty acids(mmol/100g fat) ³	2.13 ^b	0.99 ^a	1.60 ^{ab}	0.284	0.036

¹Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

²SEM for *n*=14.

³ Measured after storage at 4°C for 34h post-milking.

^{a, b, c}Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

702 **Table 4** Effect of dietary supplements of extruded linseeds alone or in combination with fish
703 oil on milk fatty acid composition in lactating goats.

Fatty acid (g/100 g fatty acids)	Treatment ¹			SEM ²	P
	Control	EL	ELFO		
4:0	1.81 ^b	1.94 ^a	2.03 ^a	0.0626	0.049
6:0	2.25 ^a	1.98 ^b	2.15 ^{ab}	0.0637	0.015
7:0	0.047 ^b	0.058 ^a	0.066 ^a	0.0038	0.006
8:0	2.67 ^a	2.11 ^b	2.33 ^b	0.0970	0.002
9:0	0.10	0.11	0.12	0.0082	0.139
10:0	10.90 ^a	6.99 ^c	8.02 ^b	0.3280	<0.001
<i>cis</i> -9 10:1	0.26	0.14 ^b	0.15 ^b	0.0103	<0.001
11:0	0.18	0.15	0.19	0.0152	0.293
12:0	6.19 ^a	3.24 ^c	4.09 ^b	0.1812	<0.001
<i>cis</i> -9 12:1	0.18	0.16	0.17	0.0162	0.625
<i>trans</i> -9 12:1	0.022 ^a	0.014 ^b	0.023 ^a	0.0021	0.024
13:0	0.12 ^a	0.04 ^b	0.07 ^b	0.0129	<0.001
13:0 <i>iso</i>	0.028 ^a	0.011 ^b	0.016 ^b	0.0020	<0.001
13:0 <i>anteiso</i>	0.078 ^a	0.033 ^c	0.046 ^b	0.0029	<0.001
14:0	12.98 ^a	7.31 ^c	9.55 ^b	0.2725	<0.001
14:0 <i>iso</i>	0.11 ^a	0.08 ^b	0.07 ^b	0.0067	<0.001
<i>cis</i> -9 14:1	0.23 ^a	0.09 ^c	0.13 ^b	0.0089	<0.001
<i>trans</i> -9 14:1	0.001 ^b	0.010 ^b	0.030 ^a	0.0030	<0.001
15:0	1.05 ^a	0.80 ^b	1.02 ^a	0.0450	0.002
15:0 <i>anteiso</i>	0.46 ^a	0.33 ^c	0.39 ^b	0.0157	<0.001
15:0 <i>iso</i>	0.27 ^a	0.15 ^c	0.22 ^b	0.0131	<0.001
<i>trans</i> -5 15:1	0.032 ^b	0.021 ^c	0.042 ^a	0.0027	<0.001
16:0	29.60 ^a	16.89 ^c	21.79 ^b	0.9487	<0.001
16:0 <i>iso</i>	0.37 ^a	0.24 ^b	0.25 ^b	0.0180	<0.001
<i>cis</i> -9 16:1	0.69 ^a	0.36 ^b	0.68 ^a	0.0442	<0.001
<i>cis</i> -11 16:1	0.014 ^a	0.007 ^b	0.018 ^a	0.0017	<0.001
<i>cis</i> -9, <i>cis</i> -13 16:2	tr ^c	0.023 ^b	0.068 ^a	0.0073	<0.001
<i>trans</i> -9 16:1	0.09 ^b	0.20 ^b	0.51 ^a	0.0520	<0.001
<i>trans</i> -10 16:1	0.006 ^c	0.033 ^b	0.094 ^a	0.0049	<0.001
<i>trans</i> -11 16:1	0.02 ^b	0.14 ^a	0.22 ^a	0.0394	0.008
<i>trans</i> -12 16:1 ³	0.22 ^b	0.28 ^a	0.24 ^b	0.0106	0.002
17:0	0.56	0.48	0.60	0.0469	0.213
17:0 <i>iso</i> ⁴	0.53 ^a	0.41 ^b	0.58 ^a	0.0212	<0.001
17:0 <i>anteiso</i>	0.60	0.46	0.56	0.0431	0.105
<i>cis</i> -9 17:1	0.25 ^a	0.15 ^b	0.15 ^b	0.0070	<0.001
18:0	5.53 ^b	14.76 ^a	4.94 ^b	0.7494	<0.001
18:0 <i>iso</i>	0.046 ^a	0.010 ^b	0.004 ^b	0.0030	<0.001
10-oxo 18:0	0.003 ^b	0.074 ^b	0.440 ^a	0.0424	<0.001
18:3 n-3	0.62 ^c	2.19 ^a	1.24 ^b	0.1006	<0.001
<i>cis</i> -9, <i>trans</i> -11, <i>trans</i> -15 18:3 ⁵	0.014 ^b	0.036 ^b	0.091 ^a	0.0107	<0.001
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.008 ^c	0.150 ^a	0.068 ^b	0.0129	<0.001
19:0	0.039	0.042	0.044	0.0073	0.893

20:0	0.12 ^b	0.14 ^a	0.18 ^a	0.0180	0.080
<i>cis</i> -11 20:1	0.044 ^b	0.039 ^b	0.247 ^a	0.0130	<0.001
20:4n-3	tr ^b	0.001 ^b	0.042 ^a	0.0038	<0.001
20:4n-6	0.101 ^a	0.068 ^b	0.069 ^b	0.0045	<0.001
20:5n-3	0.085 ^b	0.101 ^b	0.146 ^a	0.0065	<0.001
22:0	0.031 ^b	0.033 ^b	0.058 ^a	0.0047	<0.001
<i>cis</i> -11 22:1	tr ^b	tr ^b	0.167 ^a	0.0130	<0.001
22:2n-6	0.022 ^a	0.014 ^b	0.020 ^a	0.0016	0.010
22:4n-3	0.002 ^b	0.013 ^b	0.067 ^a	0.0055	<0.001
22:5n-3	0.121 ^b	0.107 ^b	0.167 ^a	0.0081	<0.001
22:6n-3	0.049 ^b	0.031 ^b	0.098 ^a	0.0068	<0.001
23:0	0.004 ^b	0.001 ^b	0.019 ^a	0.0015	<0.001
Sum of fatty acids					
Σ <i>trans</i> -18:1	1.72 ^c	5.82 ^b	13.72 ^a	1.0366	<0.001
Σ <i>cis</i> -18:1	14.15 ^b	25.35 ^a	12.32 ^b	0.9350	<0.001
Σ 18:2 ⁶	2.76 ^c	3.88 ^b	4.80 ^a	0.3014	<0.001
Σ CLA	0.530 ^b	0.991 ^b	2.725 ^a	0.3171	<0.001
Σ saturates	76.15 ^a	58.48 ^b	59.33 ^b	1.0542	<0.001
Σ MUFA	17.93 ^c	32.81 ^a	28.91 ^b	0.7750	<0.001
Σ PUFA	4.31 ^c	7.60 ^b	9.60 ^a	0.5482	<0.001
Ratio of fatty acids					
Σn-3/ Σn-6	0.35 ^b	1.20 ^a	1.18 ^a	0.0336	<0.001
18:3n3/18:2n6	0.26 ^c	1.12 ^a	0.88 ^b	0.0312	<0.001
<i>cis</i> -9 14:1/14:0	0.018 ^a	0.012 ^c	0.014 ^b	0.0007	<0.001
<i>cis</i> -9 16:1/16:0	0.023 ^b	0.022 ^b	0.031 ^a	0.0013	<0.001
<i>cis</i> -9 18:1/18:0	2.517 ^a	1.580 ^b	2.762 ^a	0.1370	<0.001
<i>cis</i> -9, <i>trans</i> -11 18:2/ <i>trans</i> -11 18:1	0.615 ^a	0.437 ^b	0.441 ^b	0.0252	<0.001

¹Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

²SEM for *n*=14.

³Contains *trans*-6,+ -7 + -8 16:1 as a minor component

⁴Contains *cis*-7 16:1 as a minor component.

⁵Contains *trans*-13,*cis*-17 20:2 as a minor component.

⁶Sum of 18:2 fatty acids excluding isomers of CLA.

^{a, b, c} Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

CLA, conjugated linoleic acid.

MUFA, monounsaturated fatty acids.

PUFA, polyunsaturated fatty acids.

tr: indicates concentrations below 0.001 g/100 g fatty acids.

Table 5 Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on milk 18:1 composition in lactating goats.

Fatty acid (g/100 g fatty acids)	Treatment ¹			SEM ²	P
	Control	EL	ELFO		
<i>cis</i> -9 18:1 ³	13.43 ^b	22.82 ^a	10.80 ^b	0.9145	<0.001
<i>cis</i> -11 18:1	0.35 ^b	0.45 ^b	0.73 ^a	0.0444	<0.001
<i>cis</i> -12 18:1	0.13 ^b	0.33 ^a	0.11 ^b	0.0195	<0.001
<i>cis</i> -13 18:1	0.028 ^b	0.094 ^a	0.066 ^a	0.0099	<0.001
<i>cis</i> -14 18:1 ⁴	0.12 ^c	0.88 ^a	0.35 ^b	0.0552	<0.001
<i>cis</i> -15 18:1 ⁵	0.08 ^b	0.79 ^a	0.26 ^b	0.0767	<0.001
<i>cis</i> -16 18:1 ⁶	0.04 ^c	0.19 ^a	0.12 ^b	0.0152	<0.001
<i>trans</i> -4 18:1	0.001 ^b	0.022 ^a	0.015 ^a	0.0037	0.002
<i>trans</i> -5 18:1	0.002 ^b	0.020 ^a	0.017 ^a	0.0036	0.005
<i>trans</i> -6 +-7 +-8 18:1	0.08 ^c	0.32 ^b	0.48 ^a	0.0487	<0.001
<i>trans</i> -9 18:1	0.14 ^c	0.31 ^b	0.55 ^a	0.0393	<0.001
<i>trans</i> -10 18:1	0.33 ^b	0.81 ^b	5.42 ^a	0.9912	0.002
<i>trans</i> -11 18:1	0.76 ^b	1.88 ^b	5.39 ^a	0.5772	<0.001
<i>trans</i> -12 18:1 ⁷	0.14 ^b	0.62 ^a	0.77 ^a	0.0546	<0.001
<i>trans</i> -13 +-14 18:1	0.26 ^c	1.83 ^a	1.07 ^b	0.1601	<0.001

¹Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

²SEM for *n*=14

³Contains *cis*-10-18:1, and *trans*-15 18:1 as minor components.

⁴Contains *trans*-16 18:1 as a minor component.

⁵Contains *trans*-17 18:1 as a minor component.

⁶Contains *cis*-9, *trans*-12 18:2 as a minor component.

⁷Contains *cis*-6 + -7 +-8 18:1 as a minor component.

^{a, b, c} Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

tr: indicates concentrations below 0.001 g/100 g fatty acids

Table 6 Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on milk 18:2 composition (mg/100g total fatty acids) in lactating goats.

Isomer (mg/100 g fatty acids)	Treatment ¹			SEM ²	P
	Control	EL	ELFO		
<i>cis</i> -9, <i>trans</i> -12 18:2 ³	59 ^c	338 ^a	169 ^b	24.48	<0.001
<i>cis</i> -9, <i>trans</i> -13 18:2	151 ^b	682 ^a	563 ^a	41.45	<0.001
<i>trans</i> -9, <i>trans</i> -12 18:2	11 ^b	35 ^b	325 ^a	64.36	0.002
<i>trans</i> -11, <i>cis</i> -15 18:2	134 ^b	722 ^b	2208 ^a	239.97	<0.001
<i>cis</i> -9, <i>cis</i> -12 18:2	2340 ^a	1900 ^b	1390 ^c	76.44	<0.001
<i>cis</i> -9, <i>trans</i> -11 CLA	467 ^b	787 ^b	2502 ^a	313.79	<0.001
<i>cis</i> -11, <i>trans</i> -13 CLA	1 ^c	2 ^a	1 ^b	0.23	<0.001
<i>cis</i> -12, <i>trans</i> -14 CLA	1 ^b	2 ^a	1 ^b	0.18	<0.001
<i>trans</i> -7, <i>cis</i> -9 CLA	23 ^b	57 ^a	70 ^a	7.03	<0.001
<i>trans</i> -8, <i>cis</i> -10 CLA	7 ^a	5 ^a	2 ^b	1.36	0.055
<i>trans</i> -9, <i>cis</i> -11 CLA	4 ^b	10 ^b	58 ^a	9.22	<0.001
<i>trans</i> -10, <i>cis</i> -12 CLA	2	3	2	0.56	0.584
<i>trans</i> -11, <i>cis</i> -13 CLA	6 ^b	34 ^a	26 ^a	5.43	0.004
<i>trans</i> -12, <i>cis</i> -14 CLA ⁴	2 ^b	23 ^a	5 ^b	2.81	<0.001
<i>trans</i> -6, <i>trans</i> -8 CLA	tr ^b	tr ^b	1 ^a	0.24	0.012
<i>trans</i> -7, <i>trans</i> -9 CLA	1 ^b	1 ^b	4 ^a	0.30	<0.001
<i>trans</i> -8, <i>trans</i> -10 CLA	2 ^b	2 ^b	9 ^a	0.65	<0.001
<i>trans</i> -9, <i>trans</i> -11 CLA	7 ^b	11 ^b	19 ^a	1.67	<0.001
<i>trans</i> -10, <i>trans</i> -12 CLA	2 ^b	3 ^b	6 ^a	0.77	<0.001
<i>trans</i> -11, <i>trans</i> -13 CLA	4 ^b	26 ^a	7 ^b	2.21	<0.001
<i>trans</i> -12, <i>trans</i> -14 CLA	2 ^c	23 ^a	11 ^b	2.05	<0.001
<i>trans</i> -13, <i>trans</i> -15 CLA	1 ^c	2 ^a	1 ^b	0.19	<0.001

¹Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

²SEM for $n=14$.

³Contains *cis*-9,*trans*-14 18:2 as a minor component.

⁴Contains *cis*-13,*trans*-15 CLA as a minor component.

^{a, b, c} Mean values for each treatment within a row not sharing a common superscript differ ($P<0.05$). CLA, conjugated linoleic acid.

Table 7 Pearson correlation coefficients between milk fat content (g/kg) and concentrations of specific fatty acids in milk (g/100g of fatty acids) in goats fed grass hay based diets containing no additional lipid, extruded linseeds alone or in combination with fish oil. Relationships derived using 42 measurements made for 14 animals¹

	Fat content	<i>cis</i> -11 16:1	<i>cis</i> -11 18:1	<i>cis</i> -11 20:1	<i>trans</i> -10 18:1	<i>trans</i> -9, <i>cis</i> - 11 CLA	<i>trans</i> - 8, <i>trans</i> -10 CLA	<i>trans</i> - 7, <i>trans</i> -9 CLA	<i>trans</i> - 10, <i>trans</i> - 12 CLA
<i>cis</i> -11 16:1	-0.682 ^{***}								
<i>cis</i> -11 18:1	-0.581 ^{***}	+0.585 ^{***}							
<i>cis</i> -11 20:1	-0.533 ^{***}	+0.626 ^{***}	+0.864 ^{***}						
<i>trans</i> -10 18:1	-0.476 ^{**}	+0.532 ^{***}	+0.781 ^{***}	+0.756 ^{***}					
<i>trans</i> -9, <i>cis</i> -11 CLA	-0.503 ^{***}	+0.573 ^{***}	+0.834 ^{***}	+0.511 ^{***}	+0.964 ^{***}				
<i>trans</i> -8, <i>trans</i> -10 CLA	-0.597 ^{***}	+0.510 ^{***}	+0.875 ^{***}	+0.602 ^{***}	+0.630 ^{***}	+0.670 ^{***}			
<i>trans</i> -7, <i>trans</i> -9 CLA	-0.607 ^{***}	+0.531 ^{***}	+0.778 ^{***}	+0.862 ^{***}	+0.599 ^{***}	+0.643 ^{***}	+0.922 ^{***}		
<i>trans</i> -10, <i>trans</i> -12 CLA	-0.460 ^{**}	+0.490 ^{***}	+0.794 ^{***}	+0.728 ^{***}	+0.923 ^{***}	+0.884 ^{***}	+0.679 ^{***}	+0.613 ^{***}	
18:0	+0.606 ^{***}	-0.698 ^{***}	-0.358 [*]	-0.551 ^{***}	-0.387 [*]	-0.402 ^{**}	-0.486 ^{***}	-0.608 ^{***}	-0.294

¹Signs indicate the effect of the variable on the predictor

CLA: conjugated linoleic acid

^{*}, ^{**}, ^{***} $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Figure 1

